BIOINFORMATICS DISCOVERY NOTE
Vol. 25 no. 4 2009, pages 430–434
doi:10.1093/bioinformatics/btn646

Gene expression

Computational analysis of microRNA profiles and their target genes suggests significant involvement in breast cancer antiestrogen resistance

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Received August 7, 2008; revised December 9, 2008; accepted December 12, 2008

Advance Access publication December 17, 2008

Associate Editor: Ivo Hofacker

ABSTRACT

Motivation: Recent evidence shows significant involvement of microRNAs (miRNAs) in the initiation and progression of numerous cancers; however, the role of these in tumor drug resistance remains unknown.

Results: By comparing global miRNA and mRNA expression patterns, we examined the role of miRNAs in resistance to the 'pure antiestrogen' fulvestrant, using fulvestrant-resistant MCF7-FR cells and their drug-sensitive parental estrogen receptor (ER)-positive MCF7 cells. We identified 14 miRNAs downregulated in MCF7-FR cells and then used both TargetScan and PITA to predict potential target genes. We found a negative correlation between expression of these miRNAs and their predicted target mRNA transcripts. In genes regulated by multiple miRNAs or having multiple miRNA-targeting sites, an even stronger negative correlation was found. Pathway analyses predicted these miRNAs to regulate specific cancer-associated signal cascades. These results suggest a significant role for miRNA-regulated gene expression in the onset of breast cancer antiestrogen resistance, and an improved understanding of this phenomenon could lead to better therapies for this often fatal condition.

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 INTRODUCTION

MicroRNAs (miRNAs) are small, non-coding RNAs that have been shown to influence the stability and translational efficiency of cognate mRNAs (Farh *et al*., 2005; Lim *et al*., 2005). MiRNAs are known to control diverse biological processes, and recent studies have shown dysregulation of numerous miRNAs in the initiation and progression of various cancers (Cimmino *et al*., 2005; Eis *et al*., 2005; Hayashita *et al*., 2005; Iorio *et al*., 2005; Johnson *et al*., 2005; Ma *et al*., 2007; Takamizawa *et al*., 2004), allowing miRNA expression profiles to be potentially used for cancer classification, diagnosis and prognosis (Calin and Croce, 2006; Lu *et al*., 2005), and specific miRNAs could represent therapeutic targets (Hernando, 2007; Negrini *et al*., 2007). In human breast cancer, miRNAs -let7i, -125b, 145, 21, 155 and 191 are significantly downregulated, as compared with normal breast tissue (Foekens *et al*., 2008; Iorio *et al*., 2005). Furthermore, altered expression of specific miRNAs could be associated with poor prognosis, e.g. let-7 (Iorio *et al*., 2005), 212 (Iorio *et al*., 2005), 181 (Iorio *et al*., 2005; Volinia *et al*., 2006) and 191 (Foekens *et al*., 2008), and miR-10b can specifically initiate invasion and metastasis (Ma *et al*., 2007). The importance of miRNAs in these advanced breast cancer phenotypes raises the question of their further involvement in antiestrogen resistance.

Fulvestrant (Faslodex; ICI 182780), an advanced breast cancer therapy belonging to a new class of antihormonal agents known as selective estrogen receptor downregulators (SERDs) (Howell, 2000), is FDA approved for use in postmenopausal patients following failure of first-line endocrine therapies (such as tamoxifen), with a markedly different mechanism of action. Specifically, this 'pure estrogen antagonist' inhibits cytoplasm-to-nucleus translocation, dimerization and DNA binding of the estrogen receptor-alpha (ERα), while also inducing ERα cytoplasmic aggregation, immobilization to the nuclear matrix, and proteasomal degradation (Fan *et al*., 2003; Long and Nephew, 2006). As a consequence of these actions, loss of both ERα-mediated genomic and non-genomic pathways leads to the complete suppression of ERα signaling (McDonnell, 2005). Despite these potent antiestrogen effects, most breast cancers eventually develop resistance to

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fulvestrant (Howell, 2001; Howell and Abram, 2005), through poorly understood mechanisms.

To investigate possible molecular changes associated with the development of antiestrogen resistance, we previously subjected fulvestrant-resistant breast cancer cells to global gene expression microarray analyses (Fan *et al*., 2006). From these studies, we discovered antiestrogen resistance to be associated with autocrine-induced proliferation likely due to dysregulated EGFR, ErbB2, cytokines/cytokine receptors, Wnt/β-catenin, Notch and IFN-signaling pathways. These previous discoveries in MCF7-FR cells strongly support the value of this cell line as a model system for studying antiestrogen resistance in human breast cancer. In the present study, we combined global gene and miRNA expression array data to further examine a potential role for miRNAs in the onset of this devastating condition.

2 METHODS

2.1 Cell culture and RNA isolation

ER-positive MCF7 cells and their fulvestrant-resistant daughter MCF-FR cells were cultured as previously described (Fan *et al*., 2006). For gene expression studies, total RNA was isolated using RNeasy Mini Kits (Qiagen, Valencia, CA, USA), converted to cRNA, labeled and hybridized to Affymetrix U133 Plus 2.0 arrays (Affymetrix, Santa Clara, CA, USA) by the Indiana University Center for Medical Genomics. For microRNA isolation, pelleted cells were resuspended in TRIzol reagent (Invitrogen, Carlsbad, CA, USA) (which retains small RNA molecules), subjected to polyethylene glycol precipitation, RNA ligase-mediated labeling, and hybridized to our custom array, according to our previously published method (Thomson *et al*., 2004).

2.2 Microarray analysis of miRNA and mRNA in MCF7 and MCF7-FR cells

Genome-wide mRNA expression was assessed using *Affymetrix Human Genome U133 Plus 2.0* microarrays (Fan *et al*., 2006). Average hybridization signal intensities from four replicates were used for data analysis, and genes with signal density <300 pixels were excluded. Moderated *t*-statistics were calculated by LIMMA (Bioconductor), with $P < 0.05$ considered significant. Up- or downregulated genes were, respectively, defined as those with at least 2-fold increased or decreased signal intensity. A custom microarray (Thomson *et al*., 2004) was used to determine miRNA expression, using two replicates for each cell line. Clustering of miRNA expression data was performed using CLUSTER (Eisen *et al*., 1998), with filtering to remove inconsistencies between replicates. For clustering, we first log-transformed the data and median-centered the array and genes, followed by average linkage clustering. Clustering results were visualized by TREEVIEW (http://rana.lbl.gov/EisenSoftware.htm).

Student's *t*-test was performed to evaluate the statistical significance of the cluster selection. The results showed that out of 16 microRNAs identified by our clustering methods and across all probes analyzed, 20 probes were among the top 22 smallest *P*-values ($P < 0.05$; Supplementary Table 1).

2.3 MiRNA target analysis

For comprehensive prediction of miRNA target genes, two publically available algorithms (each using dissimilar methods of target identification) were used, the recently described probability of interaction by target accessibility (PITA) (Kertesz *et al*., 2007), and TargetScan, release 4.2 (www.targetscan.org) (Lewis *et al*., 2005). PITA was used on the prediction results for all identified miRNAs in the human genome with criteria '3/15 flank' (downloaded from http://genie.weizmann.ac.il/pubs/mir07/mir07_data.html). We further used a $\Delta\Delta G < 0$ cutoff to filter the prediction results. $\Delta\Delta G$ scores are computed as the free energy gained by microRNA to target binding (Kertesz *et al*., 2007). Only targets identified by both TargetScan and PITA were considered to be the true targets for each miRNA. As no target genes were identified for hsa-miR-373* (miR-373* is not listed in those databases), TargetScan and PITA online prediction algorithms were used. Biological pathway analyses were performed using Pathway-Express (Draghici *et al*., 2007).

3 RESULTS

3.1 Differentially expressed miRNAs in MCF7-FR compared with MCF7 cells

To study the role of miRNA-mediated gene regulation in fulvestrant resistance, we compared miRNA expression in MCF7-FR versus MCF7 cells. We then analyzed the differential miRNA expression profiles to identify three clusters of miRNAs: (i) a downregulated group (14 miRNAs) (Fig. 1A); (ii) an upregulated group (two miRNAs) (Fig. 1B); and (iii) an unchanged group (data not shown).

The potential target genes of these 14 MCF7-FR-downregulated miRNAs were then identified based on overlap between TargetScan and PITA. For these 14 downregulated miRNAs, we hypothesized the mRNA levels of their target genes to be upregulated (Farh *et al*., 2005; Lim *et al*., 2005). Based on the overlap between TargetScan and PITA target prediction, we determined the number of potential target genes upregulated (fold change >2, $P < 0.05$, FR versus MCF7) and not-upregulated. Out of a total of 19 886 genes on the microarray, 3297 total target genes were found upregulated in MCF7-FR cells, 1895 were predicted to be regulated by any of the 14 downregulated miRNAs. Of these 1895 genes, 587 were upregulated, and this ratio is significant ($P = 1.45E\text{-}60$, Fisher's exact test). The total numbers of genes predicted as targets for each of the 14 downregulated miRNAs are listed in Table 1, accompanied by the actual number of genes upregulated in MCF7-FR. By comparing the number of miRNA-upregulated genes with the total number of genes up- and not-upregulated, we discovered that 13 of the 14 miRNAs downregulated in MCF7-FR demonstrated significantly higher ratios of upregulated genes than predicted (Table 1). This strong inverse correlation between miRNA and target

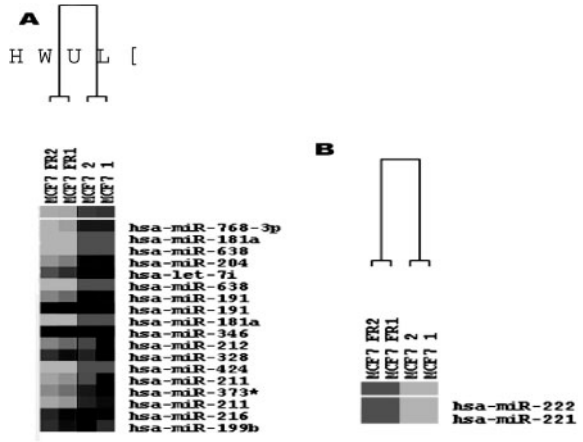

Fig. 1. Clustering of miRNA array results comparing MCF7-FR with MCF7 cells identified two separate groups having (**A**) downregulated or (**B**) upregulated expression in MCF7-FR cells. Grey signifies lower signal intensity, while black denotes higher signal intensity.

Table 1. Fisher's exact test of miRNA target genes upregulated in MCF7-FR cells

miRNA	Number of targets upregulated in FR cells	Number of targets not-upregulated in FR cells	*P*-value
hsa-let-7i	130	344	1.28E-09*
hsa-miR-181a	153	268	3.55E-23*
hsa-miR-191	4	17	4.69E-01
hsa-miR-199b	54	106	8.10E-08*
hsa-miR-204	63	130	2.95E-08*
hsa-miR-211	57	130	1.64E-06*
hsa-miR-212	51	104	4.49E-07*
hsa-miR-216	27	57	3.31E-04*
hsa-miR-328	21	63	3.10E-02*
hsa-miR-346	23	41	1.41E-04*
hsa-miR-373*	100	168	1.77E-16*
hsa-miR-424	133	281	3.02E-15*
hsa-miR-638	11	27	4.01E-02*
hsa-miR-768-3p	77	94	2.15E-18*

'Number of targets upregulated in MCF7-FR cells' refers to the number of target genes upregulated in MCF7-FR cells. 'Number of targets not-upregulated in FR cells' refer to the number of target genes not-upregulated in MCF7-FR cells.
*$P < 0.05$ (one-tailed Fisher's exact test).

gene expression suggests that these miRNAs play a significant role in gene regulation during the acquisition of fulvestrant resistance.

For the upregulated miRNA cluster, of 163 genes predicted as targets of miR-221 or miR-222, 20 were found downregulated, compared with 1950 (of the 19 886 total) MCF7-FR-downregulated genes. However, as the number of genes downregulated by miR-221 or miR-222 was not significant ($P = 0.17$), these miRNAs may preferentially mediate translational repression, rather than mRNA degradation (Bagga *et al*., 2005).

3.2 Genes targeted by multiple downregulated miRNAs are more likely to be upregulated than genes targeted by a single miRNA

To further analyze the role of miRNAs in gene regulation in fulvestrant-resistant cells, we examined whether gene-specific regulation correlated to the number of miRNA target sites. Specifically, we separated the target genes of the 14 downregulated miRNAs into two groups: (i) those regulated by only one of these 14 miRNAs; or (ii) those regulated by multiple miRNAs. A one-tailed Fisher's exact test was then performed to determine whether multiple miRNA regulation would further improve the Up/Not-up upregulated gene ratio. The results showed that possible targeting by two or more downregulated miRNAs resulted in a significantly higher ratio of upregulated genes, as compared with targeting by only one downregulated miRNA (Table 2), suggesting more efficient gene regulation by multiple miRNAs acting in collaboration.

3.3 Genes possessing multiple downregulated miRNA target sites are more likely to be upregulated than those with only one site

To investigate the effect of multiple miRNA binding sites, we compared the ratio of upregulated genes among genes having single target sites (356 out of 1265 genes) to the ratio of upregulated genes among that having multiple target sites (231 out of 630 genes). The results (Table 2) show that multiple miRNA targeting sites resulted in a significantly higher ratio of upregulated genes, as compared with only one target site. This result is consistent with our findings from the previous section, providing further evidence for the collaboration among multiple miRNAs in gene regulation.

Table 2. Comparisons of multiple versus single miRNA regulation, and genes having multiple versus single target sites

Conditions	Up	Not-Up	Ratio	*P*-value
Single miRNA regulation	375	947	0.396	2.32E-04*
Multiple miRNAs regulation	212	361	0.587	
Single targeting site	356	909	0.392	1.80E-04*
Multiple targeting sites	231	399	0.579	

'Up' means the actual number of genes upregulated in MCF7-FR cells. 'Not-Up' is the total number of predicted gene targets of the 14 miRNAs; 'Ratio' is the ratio between actual/predicted upregulated genes (up/total).
*$P < 0.05$ (one-tailed Fisher's exact test).

3.4 Pathway analysis predicts that downregulated and upregulated miRNAs regulate specific biological cascades in MCF7-FR cells

To assess the possible biological impact of the 14 downregulated miRNAs in fulvestrant-resistant cells, we performed pathway enrichment analysis of all upregulated genes (3297 genes) and upregulated targets of the 14 miRNAs (587 genes). We then compared the two pathway analysis profiles using Fisher's exact test. As shown in Supplementary Table 2, miRNA targets were found to be enriched in 13 of the 19 pathways significantly altered in MCF7-FR cells, including well-described signaling pathways such as TGF-β, Wnt, MAPK signaling and mTOR. Supplementary Table 3 lists the predicted target genes corresponding to the different pathways.

Differential upregulation of a group of two microRNAs, miR-221/222, was observed in the MCF7-FR versus MCF7 cells. Pathway analysis for the 20 target genes predicted to be downregulated (see above) suggests that miR221/222 target the ErbB signaling pathway. Our gene expression microarray analysis supports this possibility, showing significantly decreased expression of ErbB and another member of the ErbB pathway, the cell-cycle inhibitor p27/^{Kip1}, in MCF7-FR cells (Fan *et al*., 2006 and our unpublished observations).

4 DISCUSSION

Recent studies have shown numerous miRNAs to be dysregulated in various cancers, and consequently, we compared mRNA and miRNA expression profiles to examine the role of miRNA in the development of antiestrogen-resistant breast cancer. The involvement of miRNAs in chemotherapy resistance has been recently suggested (Blower *et al*., 2008; Meng *et al*., 2006; Salter *et al*., 2008; Xia *et al*., 2008; Yang *et al*., 2008), and to our knowledge, this is the first report of an association between miRNAs and acquired resistance to fulvestrant, the second-line drug given to postmenopausal women with ER-positive, tamoxifen-resistant tumors. Our results further suggest that miRNA-regulated gene expression can be detected at

the mRNA level, a finding consistent with previous reports (Farh *et al*., 2005; Lim *et al*., 2005). However, two recent studies further substantiate the impact of microRNAs at the level of translation (Baek *et al*., 2008; Selbach *et al*., 2008), and although our method does not account for miRNA regulation by translational repression, it does provide a computational approach for using gene expression microarray data to study miRNA regulation, a highly feasible and straight-forward approach, given the abundance of such studies and the relative paucity of corresponding proteomics data.

Using a custom microarray (Thomson *et al*., 2004), we identified 14 downregulated but only two upregulated miRNAs in MCF7-FR cells, consistent with a previous study showing that miRNA tend to be preferentially downregulated in cancer (Meng *et al*., 2006). Of the 14 downregulated miRNAs, 11 have been previously associated with various cancers (let-7i, miR-181a, 191, 199b, 204, 211, 212, 216, 328, 373*, 424), of which three (miR-204, 191 and let-7i) have been specifically linked to breast cancer (Jiang *et al*., 2008). Our results further suggest that these 14 miRNAs have potential relevance to the acquisition of fulvestrant resistance, including three miRNAs previously unreported in breast cancer (miR-346, 638 and 768-3p). The identification of miRNAs that are differentially expressed in fulvestrant-resistant cell lines could serve as potential biomarkers of fulvestrant-resistant tumors. Moreover, determination of the target genes/pathways of these dysregulated miRNAs will further enhance our knowledge of fulvestrant resistance and facilitate design of new targeted therapeutic agents that might allow for the prevention or reversal of resistance to this agent. For example, therapeutic strategies aimed at blocking expression of these miRNAs in antiestrogen-*sensitive* breast cancer may allow for a greater response to fulvestrant therapy in a subset of breast cancers. In addition, altered expression of these miRNAs themselves could be predictive of drug resistance development and prove to be valuable markers in the personalized clinical management of breast cancer.

Our pathway analyses suggest involvement of miRNA in regulation of biologically important signaling cascades, including TGF-β (Supplementary Table 2). Significant experimental evidence suggests that loss of growth-inhibitory response to TGF-β is associated with breast neoplasia (Benson, 2004; Muraoka-Cook *et al*., 2005). Furthermore, WNT proteins are frequently overexpressed in breast tumors (Lin *et al*., 2000), and dysregulation of this pathway is likely to have a major impact on several aspects of breast cancer biology (Li *et al*., 2003; Klarmann *et al*., 2008). As we previously demonstrated that fulvestrant-resistant breast cancer cells utilize multiple growth-stimulatory pathways to establish hormone-independence and autocrine-regulated proliferation (Fan *et al*., 2006), including a role for Wnt/β-catenin and EGFR/ErbB2 signaling pathways in estrogen-independent growth, the results of the current study suggest that miRNAs may play a significant role in this process.

In summary, we have used experimental microarray data and computational approaches to strongly implicate specific miRNAs in the development of fulvestrant resistance in breast cancer. Further elucidation of the mechanisms underlying miRNA regulation and function in breast cancer cells could provide novel therapeutic strategies against this destructive impediment to successful treatment of this common malignancy.

ACKNOWLEDGEMENTS

The authors thank Dr Seung Yoon Nam for helpful discussion. The microarray studies were carried out using the facilities of the Center for Medical Genomics at Indiana University School of Medicine. The Center for Medical Genomics is supported in part by the Indiana Genomics Initiative of Indiana University, INGEN, which is supported in part by the Lilly Endowment, Inc.

Funding: National Institute of Health grants (CA085289, CA113001); Walther Cancer Institute Foundation, Indianapolis, IN.

Conflict of Interest: none declared.

REFERENCES

Baek,D. *et al*. (2008) The impact of microRNAs on protein output. *Nature,* **455**, 64–71.

Bagga,S. *et al*. (2005) Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. *Cell,* **122**, 553–563.

Benson,J.R. (2004) Role of transforming growth factor beta in breast carcinogenesis. *Lancet Oncol*., **5**, 229–239.

Blower,P.E. *et al*. (2008) MicroRNAs modulate the chemosensitivity of tumor cells. *Mol. Cancer Ther*., **7**, 1–9.

Calin,G.A. and Croce,C.M. (2006) MicroRNA-cancer connection: the beginning of a new tale. *Cancer Res*., **66**, 7390–7394.

Cimmino,A. *et al*. (2005) miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc. Natl Acad. Sci. USA*, **102**, 13944–13949.

Draghici,S. *et al*. (2007) A systems biology approach for pathway level analysis. *Genome Res*., **17**, 1537–1545.

Eis,P.S. *et al*. (2005) Accumulation of miR-155 and BIC RNA in human B cell lymphomas. *Proc. Natl Acad. Sci. USA*, **102**, 3627–3632.

Eisen,M.B. *et al*. (1998) Cluster analysis and display of genome-wide expression patterns. *Proc. Natl Acad. Sci. USA*, **95**, 14863–14868.

Fan,M. *et al*. (2003) The NEDD8 pathway is required for proteasome-mediated degradation of human estrogen receptor (ER)-alpha and essential for the antiproliferative activity of ICI 182,780 in ERalpha-positive breast cancer cells. *Mol. Endocrinol*., **17**, 356–365.

Fan,M. *et al*. (2006) Diverse gene expression and DNA methylation profiles correlate with differential adaptation of breast cancer cells to the antiestrogens tamoxifen and fulvestrant. *Cancer Res*., **66**, 11954–11966.

Farh,K.K. *et al*. (2005) The widespread impact of mammalian microRNAs on mRNA repression and evolution. *Science*, **310**, 1817–1821.

Foekens,J.A. *et al*. (2008) Four miRNAs associated with aggressiveness of lymph node-negative, estrogen receptor-positive human breast cancer. *Proc. Natl Acad. Sci. USA*, **105**, 13021–13026.

Hayashita,Y. *et al*. (2005) A polycistronic microRNA cluster, miR-17-92, is overexpressed in human lung cancers and enhances cell proliferation. *Cancer Res.*, **65,** 9628–9632.

Hernando,E. (2007) microRNAs and cancer: role in tumorigenesis, patient classification and therapy. *Clin. Transl. Oncol*., **9**, 155–160.

Howell,A. (2000) Faslodex (ICI 182780). An oestrogen receptor downregulator. *Eur. J. Cancer*, **36** (Suppl. 4)**,** S87–S88.

Howell,A. (2001) Future use of selective estrogen receptor modulators and aromatase inhibitors. *Clin. Cancer Res*., **7**(Suppl. 12), 4402s-10s; discussion 4411s–4412s.

Howell,A. and Abram,P. (2005) Clinical development of fulvestrant ('Faslodex'). *Cancer Treat. Rev*., **31** (Suppl. 2)**,** S3–S9.

Iorio,M.V. *et al*. (2005) MicroRNA gene expression deregulation in human breast cancer. *Cancer Res*., **65**, 7065–7070.

Jiang,Q. *et al*. (2008) miR2Disease: a manually curated database for microRNA deregulation in human disease. *Nucleic Acids Res*., doi:10.1093/nar/gkn714.

Johnson,S.M. *et al*. (2005) RAS is regulated by the let-7 microRNA family. *Cell*, **120**, 635–647.

Kertesz,M. *et al*. (2007) The role of site accessibility in microRNA target recognition. *Nat. Genet*., **39**, 1278–1284.

Klarmann,G.J. *et al*. (2008) Epigenetic gene silencing in the Wnt pathway in breast cancer. *Epigenetics*, **3**, 59–63.

Lewis,B.P. *et al*. (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell*, **120**, 15–20.

Li,Y. *et al*. (2003) Evidence that transgenes encoding components of the Wnt signaling pathway preferentially induce mammary cancers from progenitor cells. *Proc. Natl Acad. Sci. USA*, **100**, 15853–15858.

Lim,L.P. *et al*. (2005) Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature*, **433**, 769–773.

Lin,S.Y. *et al*. (2000) Beta-catenin, a novel prognostic marker for breast cancer: its roles in cyclin D1 expression and cancer progression. *Proc. Natl Acad. Sci. USA,* **97**, 4262–4266.

Long,X. and Nephew,K.P. (2006) Fulvestrant (ICI 182,780)-dependent interacting proteins mediate immobilization and degradation of estrogen receptor-alpha. *J. Biol. Chem*., **281**, 9607–9615.

Lu,J. *et al*. (2005) MicroRNA expression profiles classify human cancers. *Nature*, **435**, 834–838.

Ma,L. *et al*. (2007) Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature*, **449**, 682–688.

McDonnell,D.P. (2005) The molecular pharmacology of estrogen receptor modulators: implications for the treatment of breast cancer. *Clin. Cancer Res*., **11**, 871s–877s.

Meng,F. *et al*. (2006) Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. *Gastroenterology*, **130**, 2113–2129.

Muraoka-Cook,R.S. *et al*. (2005) Dual role of transforming growth factor beta in mammary tumorigenesis and metastatic progression. *Clin. Cancer Res*., **11**, 937s–943s.

Negrini,M. *et al*. (2007) MicroRNAs in human cancer: from research to therapy. *J. Cell Sci*., **120**, 1833–1840.

Salter,K.H. *et al*. (2008) An integrated approach to the prediction of chemotherapeutic response in patients with breast cancer. *PLoS ONE*, **3**, e1908.

Selbach,M. *et al*. (2008) Widespread changes in protein synthesis induced by microRNAs. *Nature*, **455**, 58–63.

Takamizawa,J. *et al*. (2004) Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. *Cancer Res*., **64**, 3753–3756.

Thomson,J.M. *et al*. (2004) A custom microarray platform for analysis of microRNA gene expression. *Nat. Methods*, **1**, 47–53.

Volinia,S. *et al*. (2006) A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc. Natl Acad. Sci. USA*, **103**, 2257–2261.

Xia,L. *et al*. (2008) miR-15b and miR-16 modulate multidrug resistance by targeting BCL2 in human gastric cancer cells. *Int. J. Cancer*, **123**, 372–379.

Yang,H. *et al*. (2008) MicroRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN. *Cancer Res*., **68**, 425–433.